Current Perspective

Constitutive and Conditional Mutant Mouse Models for Understanding Dopaminergic Regulation of Orofacial Movements: Emerging Insights and Challenges

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Abstract. Among numerous mechanisms implicated in the regulation of orofacial movements, dopamine-containing neurons have received the most extensive study. Here we review the effects of a) constitutive knockout of D1–5 dopamine receptors and b) conditional mutations with progressive ablation of D1 receptor–expressing cells, on the topography of spontaneous and D1-like agonist–induced orofacial movements. In constitutive knockouts, D1 and D2 exert primary roles in regulating horizontal and vertical jaw movements, respectively, in opposite directions; in contrast, both D1 and D2 receptors regulate tongue protrusions and incisor chattering, in the same direction. D3 and D5 receptors play more subtle roles in regulating orofacial movements, while D4 receptors do not play any material role. Progressive loss of forebrain D1 receptor–expressing cells in CamKIIa/Cre D1Tox mutants is associated primarily with decreases in head and vibrissae movements, while progressive loss of striatal D1 receptor–expressing cells in DARPP-32/Cre D1Tox mutants is associated primarily with reductions in jaw movements and tongue protrusions but increases in head and vibrissae movements. Further application of constitutive and particularly conditional mutants may clarify further not only dopaminergic regulation of orofacial movements but also the pathophysiology of orofacial dysfunction in Huntington’s disease and Parkinson’s disease.

Keywords: dopamine D1–5 receptors, orofacial movement, constitutive knockout, conditional mutant, D1 receptor–expressing cell

1. Introduction

For all organisms, from the most primitive through to humans, interaction with the environment is essential for existence, with the nature of that interaction taking multiple forms that depend upon the position of the organism along the phylogenetic tree. One fundamental mode of interaction with the environment for essentially all organisms involves the orofacial region, via its participation in multiple physiological functions; these include consummatory behaviour, self-care, defensive and attack behaviours, vocalization and, in higher mammals, verbal as well as non-verbal communication. However, this diversity of functional roles implies that the underlying regulatory mechanisms, especially in mammals, must be of considerable complexity. For example, a basic human orofacial behaviour such as opening the jaw can have multiple contexts and intentions: it may be purposeful, as in eating, speaking, or as part of dental hygiene; it can occur involuntarily, as in yawning, dystonia, or Hunting-
ton’s disease (HD) (1, 2); alternatively, it may be impaired, as in dental-cranio-maxillofacial disorders or the dysphagia of Parkinson’s disease (3, 4). In each of these circumstances, some underlying commonality of motor pattern, involving generators for down-stream patterns of orofacial movement, is ‘sculpted’ by higher centres, such that the integrated orofacial (dys)function reflects the (patho)physiological and psychosocial context of the behaviour and its purpose. These concepts and principles have recently received extensive review (5 – 8).

Of necessity, diversity and complexity in these underlying mechanisms implicates the involvement of a broad range of neurotransmitter systems, among which the catecholamine dopamine (DA) has received the most extensive study (9, 10); other transmitter substances, particularly the amino acids γ-aminobutyric acid (GABA) and glutamate (10, 11) appear to play important, though lesser roles. For example, in Parkinson’s disease there is degeneration of the nigrostriatal DAergic pathway, with loss of stimulation of striatal DA receptors; this primary pathology is thought to underlie the orofacial motor symptoms of the disorder (3, 4). In contrast, in HD there is loss of striatal and cortical DA receptor–expressing cells, together with loss of GABA- and glutamate-containing neurons; this primary pathology is thought to underlie the involuntary orofacial movements of the disorder (1, 2). DA acts through numerous receptor subtypes for which medicinal chemistry has yet to identify a full range of appropriately selective agonists and antagonists (12). Despite this absence of the necessary pharmacological tools with which to investigate the differential functional roles of these receptor subtypes, they can be studied using mutant mice with targeted gene deletion [knockout (KO)] of each subtype, in comparison with wild-type (WT) controls (13, 14).

While conventional, constitutive/developmental KOs can be highly informative, the absence of the entity deleted throughout the body from conception involves anatomical regions other than those involved in the process(es) at issue and allows the recruitment of a variety of putative compensatory mechanisms that can influence the resulting phenotype; however, continuing advances in molecular biological techniques are also making available an increasing range of conditional mutants in which the investigator has control over the anatomical and temporal expression of the mutation (15). In this Perspective, we elaborate how a novel technique for the differential resolution and quantification of individual topographies of orofacial movement in mice (16, 17) has been applied to mutants with constitutive KO of each of the five DA receptor subtypes (D₁, D₂, D₃, D₄, and D₅) to illuminate their differential role in these processes. On this background (11, 18), we then consider how recent studies with conditional mutants, having progressive, postnatal loss of D₁ receptor–expressing cells from differing brain regions, are clarifying these processes in a manner more relevant to human neurological disease.

2. Methodological challenges in assessing orofacial movements in mice

Mice, the primary species for construction of KOs and other mutations, are amongst the smallest and most rapidly moving of laboratory animals, with attendant difficulties in observing and classifying orofacial movements in the absence of restriction. Therefore, we sought to develop and apply a new restrictor system designed specifically for mice (16, 17). By this method, five mice are lightly restricted by a Perspex collar around the neck that is attached to a horizontal platform; the mouse trunk can move freely, without grooming or locomotion. Orofacial movements are classified by direct visual observation, ‘blind’ to genotype and any treatment, as jaw movements in the vertical plane (Jv); jaw movements in the horizontal plane (Jh); tongue protrusions (Tg); rapid jaw movements with chattering of the incisors (Ct); movements of the head (Hd); and movements of the vibrissae (Vb). This procedure and categorization circumvents the use of commonly applied but imprecise, generic terms such as ‘vacuous chewing’, which is a composite of multiple elements, each of which may be regulated differentially (19).

To evaluate mice under spontaneous conditions (i.e., without drug challenge), the observer evaluates orofacial movements over a period of 3 h, during which the mouse habituates to the apparatus. When mice are assessed using this methodology, WT show a profile whereby vertical jaw movements are initially frequent but decline over subsequent habituation; horizontal jaw movements are initially infrequent but increase over subsequent habituation; tongue protrusions and incisor chattering occur at a low rate; head and vibrissae movements are initially frequent but decline over subsequent habituation (16, 17).

Additionally, given previous evidence that the D₁-like family of DA receptors (D₁, D₃) appear to play a more prominent role in the regulation of orofacial movements than their D₂-like counterparts (D₂, D₃, D₄) (9, 13), mice are habituated to the apparatus for 3 h and then evaluated for 1 h following challenge with the D₁-like receptor agonist SKF 83959 (0.016 – 0.4 mg/kg, i.p.), which shows high affinity for both D₁ and D₃ receptors but not for D₂, D₄, D₅, or non-DAergic receptors (12, 13). When mice are assessed using this methodology, WT show a profile whereby SKF 83959 induces vertical but not horizontal
3. Differential roles of D₁–5 receptors in orofacial movements studied using constitutive KO

3.1. Distribution of DA receptor subtypes

Among the D₁-like receptors, D₁ receptors are expressed most abundantly in the striatum and cortex, while D₅ receptors are expressed at a low density in subcortical regions such as the hippocampus and hypothalamus. Among the D₂-like receptors, D₂ receptors are also expressed most abundantly in the striatum, together with the nucleus accumbens, but less densely in extrastriatal regions; D₃ receptors are expressed most abundantly in the nucleus accumbens, olfactory tubercle, and hypothalamus, while D₄ receptors are expressed at a low density in the frontal cortex and some subcortical regions (13, 14).

3.2. D₁-like receptors

Regarding spontaneous orofacial movements (Table 1), while D₁ KO show little overall change in Jv, there is marked reduction in Jh, with slight reduction in low baseline levels of Tg and Ct and little change in Hd and Vb; interestingly, some of these overall effects are accompanied by subtle changes in habituation that are described in detail elsewhere (18, 20). Regarding orofacial movements following drug challenge, D₁ KO show marked reduction in induction of Jv, Tg, and Ct by SKF 83959, with little change in induction of Hd and Vb (20).

In D₅ KO, spontaneous Jv is increased, while Jh and Vb are decreased (Table 1); as above, some of these overall effects are accompanied by subtle changes in habituation that are described in detail elsewhere (18, 21). D₅ KO is without effect on orofacial responsivity to SKF 83959 (21).

3.3. D₂-like receptors

Regarding spontaneous orofacial movements (Table 1), D₂ KO show a small increase in Jv, with no alteration in Jh, consistently low levels of Tg and Ct, and reduction in Hd but not Vb; as for D₁-like receptors, some of these overall effects are accompanied by subtle changes in habituation that are described in detail elsewhere (18, 22). D₂ KO is without effect on orofacial responsivity to SKF 83959 (21).

In D₃ KO, spontaneous Ct is reduced but other topographies of orofacial movements are unaltered (Table 1); D₃ KO is without effect on orofacial responsivity to SKF 83959 (22). In D₄ KO, spontaneous orofacial movements are unaltered (Table 1); D₄ KO is without effect on orofacial responsivity to SKF 83959 (21).

4. Role of the D₁ receptor in orofacial movements studied using mutants with progressive, region-specific loss of brain D₁ receptor–expressing cells

D₁ and D₂ receptors are expressed on medium spiny neurons (MSNs) in the striatum, which constitutes a fundamental component of a cortico–striato–thalamo–cortical circuit; this involves a direct pathway, in which D₁ receptors are expressed on MSNs that project to the substantia nigra pars reticulata, and an indirect pathway, in which D₂ receptors are expressed on MSNs that project to the globus pallidus and, via the subthalamic nucleus, to the substantia nigra pars reticulata (23, 24). In HD, involuntary orofacial movements occur in association with progressive cell loss from the striatum (caudate nucleus and putamen), cerebral cortex, thalamus, and subthalamic nucleus, particularly MSNs of the striatum with associated loss of D₁ and D₂ receptors (1, 2).

To investigate the relative roles of loss of D₁ vs. D₂ receptors and striatal vs. extrastriatal brain regions in orofacial movement disorder of HD, we studied (25) the orofacial phenotype of a DARPP-32/Cre D₁Tox mutant line having progressive, striatal MSN-specific loss of D₁ receptor–expressing cells in comparison with CamKIIα/Cre D₁Tox mutants having progressive, generalized loss of D₁ receptor–expressing cells from the forebrain. These
two lines were generated by mating DARPP-32 or CamKIIα promoter–driven, Cre-expressing transgenic mice, respectively, with transgenic mice containing an attenuated diphtheria toxin A-chain gene down stream of a LoxP flanked NEOSTOP cassette (26, 27).

4.1. CamKIIα/Cre D1Tox mutants

Regarding spontaneous orofacial movements (Table 1), CamKIIα/Cre D1Tox mutants show little overall change in Jv, Jh, Tg, and Ct, with decreases in Hd and Vb (25). Regarding orofacial movements following drug challenge, CamKIIα/Cre D1Tox mutants show reductions in the effects of SKF 83959 on Tg, Hd, and Vb, with little change in effects on Jv, Jh, and Ct (25).

4.2. DARPP-32/Cre D1Tox mutants

Regarding spontaneous orofacial movements (Table 1), DARPP-32/Cre D1Tox mutants show marked reduction in Jh, reduction in Tg, and increases in Hd and Vb, together with some subtle changes in habituation that are described in detail elsewhere (25). Regarding orofacial movements following drug challenge, DARPP-32/Cre D1Tox mutants show reduction in the effects of SKF 83959 on Jh and Tg, with little change in effects on Jv, CT, Hd, and Vb (25).

5. Overview and conclusions

5.1. Interpretation of studies using constitutive DA receptor subtype KO

All studies using constitutive KO are subject to a number of methodological caveats that include genetic background, developmental compensation, and sex differences, supplemented in the present context by practical problems inherent to assessment of orofacial movements in mice; these important challenges are considered in detail elsewhere (13, 18). Although these issues are not to be underestimated, the results described above and summarized in Table 1 indicate that among the five DA-receptor subtypes, D1 and D2 exert primary roles in regulating horizontal and vertical jaw movements, respectively, in opposite directions; in contrast, both D1 and D2 receptors regulate tongue protrusions and incisor chattering, in the same direction. D3 and D5 receptors play more subtle roles in regulating orofacial movements, while D4 receptors do not play any material role. These profiles are sustained by findings both on spontaneous and on D1-like agonist–induced orofacial movements, with one particular exception: the superficial paradox of D1 KO attenuating spontaneous horizontal but not vertical jaw movements, while attenuating D1-like agonist–induced vertical but not horizontal jaw movements, may reflect a complex balance between D1 receptors in differing brain regions in relation to level of tonic activation.

There are clearly additional levels of mechanistic explanation, for example, the differential involvement of cyclase- vs. non-cyclase–coupled D1-like receptors and their interactions with individual members of the D2-like family, together with the involvement of GABAergic and glutamatergic processes, as considered in detail elsewhere (10, 11, 13, 18). Here, we focus on the critical issue of the specific brain regions that might be involved in the regulation of orofacial movements. Also, in human neurological disease, receptor function is not absent from conception and is therefore not fully modeled by constitutive KO; it is more typically lost over particular prenatal time frames as a neurodegenerative process. These are challenges to which conditional mutants have recently been applied.

5.2. Interpretation of studies using conditional D1 receptor mutants

Progressive loss of forebrain D1 receptor–expressing cells in CamKIIα/Cre D1Tox mutants is associated primarily with decreases in head and vibrissae movements, that is, facial hypokinesia, while progressive loss of striatal MSN D1 receptor–expressing cells in DARPP-32/Cre D1Tox mutants is associated primarily with reductions in jaw movements and tongue protrusions but increases in head and vibrissae movements, that is, oral hypokinesia with facial hyperkinesia. The extent to which the more prominent phenotype of DARPP-32/Cre D1Tox mutants might be attenuated by extra-striatal, possibly cortical changes in CamKIIα/Cre D1Tox mutants remains to be determined.

As HD is characterized clinically by orofacial dyskinesia and pathobiologically by progressive loss of D1 DA receptor–expressing cells, initially from the striatum but extending subsequently to loss from extra-striatal regions, including the cortex, application of such conditional mutants may clarify further not only DAergic regulation of orofacial movements but also the pathophysiology of orofacial dysfunction in HD and Parkinson’s disease.

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